

Applicant : Rajesh Kumar,  
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Filed : August 27, 2001  
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Attorney's Docket No.: 07064-009002

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification, and replace the original informal drawings with formal drawings. No new matter has been added.

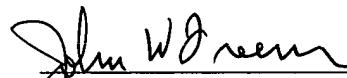
Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 07064-009002.

Respectfully submitted,

Date: \_\_\_\_\_

4/5/02

  
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**“Version With Markings to Show Changes Made”**

In the specification:

Paragraph beginning at page 12, line 7, has been amended as follows:

**Fig. 3.** DNA and protein sequence of streptokinase of *S. equisimilis* H46A (SEQ ID NOs:1 and 2, respectively) (GenBank accession number: gb/K02986/STRSKC).

Paragraph beginning at page 12, line 16, has been amended as follows:

**Fig. 6.** DNA and protein sequence of the gene-segment encoding for FBDs 1-5 of human fibronectin (SEQ ID NOs:3 and 4, respectively) (the DNA sequence has been obtained from EMBL; the file and accession no.'s are ID-HSFIBI and [X 02761,K 00799, K 02273,X 00307, X 00739] X02761, K00799, K02273, X00307, X00739).

Paragraph beginning at page 12, line 32, has been amended as follows:

**Fig. 11.** Nucleotide sequence of SK-NTRN gene (SEQ ID NO:5).

Paragraph beginning at page 12, line 34, has been amended as follows:

**Fig. 12.** Predicted secondary structure of native (A) and translationally silently modified (B) 5'-ends of the SK gene sequence (SEQ ID NOs:27 and 28, respectively).

Paragraph beginning at page 13, line 7, has been amended as follows:

**Fig. 14.** Nucleotide sequence of SK-NTR gene (SEQ ID NO:6).

Paragraph beginning at page 13, line 9, has been amended as follows:

**Fig. 15.** Schematic depiction of the intergenic region of the chimeric SK-FBD(4,5) gene (above: SEQ ID NO:8; below: SEQ ID NO:7) highlighting the design of a gly-gly-gly sequence, a transglutaminase cross-linking site and several unique restriction enzyme sites wherein different inter-genic cassettes can be conveniently swapped into this region. Also shown is the

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location of the natural Bsm I site in the SK gene which was exploited as the common junction point for joining the FBD sequences to the SK gene.

Paragraph beginning at page 13, line 23, has been amended as follows:

**Fig. 17b.** DNA sequencing data of SK-FBD(4,5) hybrid cassette in T7 expression vector, pET23(d) (SEQ ID NO:9).

Paragraph beginning at page 13, line 33, has been amended as follows:

**Fig. 19b.** DNA sequencing data of SK-FBD(1,2) hybrid cassette in T7 expression vector (SEQ ID NO:10).

Paragraph beginning at page 14, line 4, has been amended as follows:

**Fig. 21b.** DNA sequencing data of FBD(4,5)-SK gene block as present in the T7 expression vector pET23(d)-FBD(4,5)-SK (SEQ ID NO:11).

Paragraph beginning at page 14, line 10, has been amended as follows:

**Fig. 22b.** DNA sequencing data of FBD(4,5)-SK-FBD(4,5) gene block as present in the T7 expression vector pET23(d)FBD(4,5)-SK-FBD(4,5) (SEQ ID NO:12).

Paragraph beginning at page 29, line 10, has been amended as follows:

RG-7 (forward primer)

5'-ATT GCT GGA CCT GAG TGG CT-3' (SEQ ID NO:25)

(specific for the first seven codons of the SK gene; Cf Fig. 11)

Paragraph beginning at page 29, line 15, has been amended as follows:

RG-6 (reverse primer)

5'-TGG TTT TGA TTT TGG ACT-3' (SEQ ID NO:26)

(specific for codons 57-62 of SK gene)

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Paragraph beginning at page 32, line 4, has been amended as follows:

SCI-I

5'-C ATG ATA GCT GGT CCT GAA TGG CTA CTA GAT CGT CCT TCT GTA AAT  
 AAC AGC C-3' (SEQ ID NO:13)  
 (Partial NcoI site)

Paragraph beginning at page 32, line 10, has been amended as follows:

SC-II

5'-AA TTG GCT GTT ATT TAC AGA AGG ACG ATC TAG TAG CCA TTC AGG  
 ACC AGC TAT-3' (SEQ ID NO:14)  
 (Partial MfeI site)

Paragraph beginning at page 32, line 31, has been amended as follows:

Mfe I primer: 5'-C-AGC-CAA-TTG-GTT-GTT-AGC-GTT-GCT-3' (SEQ ID NO:15)

Paragraph beginning at page 37, line 24, has been amended as follows:

Forward primer (MY 13);

5'-CCG GAA TTC GCG CAA CAG ATT GTA CCC ATA GCT GAG AAG TGT TTT

Eco R1	Tansglutaminase- recognition sequence	hybridizes to upstream FBD(4,5) sequences
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GA-3' (SEQ ID NO:16)

Paragraph beginning at page 37, line 30, has been amended as follows:

Reverse primer (MY 14);

5'-GGC CTT AAG AGC GCT CTA ACG AAC ATC GGT GAA GGG GCG TCT A-3'  
 (SEQ ID NO:17)

'clamp'	Afl II	Eco 47 III	stop codon	hybridizes to downstream FBD(4,5) sequences
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'clamp'	Eco RI site	Eco 47 II	stop codons	sequence hybridizing with FBD (1,2) codons 99-104 (Cf.Fig.6)
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Paragraph beginning at page 49, line 1, has been amended as follows:

Upstream PCR-I primer KRG-8:

Transglutamate recognition site

	150    152    154
5'-CC-ATG-GTG-CAA-GCA-CAA-CAG-ATT-GTA-CCC-ATA-GCT-GAG-AAG-	
Partial Nco I	hybridizes to [begining]
site	<u>beginning</u> of FBD(4) segment
	(codon numbers of FBD are
	shown as per Fig.6)

TGT-3' (SEQ ID NO:21)

Paragraph beginning at page 49, line 9, has been amended as follows:

Downstream PCR-I primer KRG-9:

sequence complementary to codons 1-5 of SK (No.'s indicated below)

	259                      257                      255                      253
5'-CTC-AGG-TCC-AGC-AAT-ACG-AAC-ATC-GGT-GAA-GGG-GCC-AGA-T-3'	
5    4    3        2        1	

(SEQ ID NO:22)

sequence hybridizing with end of FBD(5) segment  
 (No.'s indicated are codons, as per Fig.6).

Paragraph beginning at page 49, line 21, has been amended as follows:

Upstream PCR-II primer, KRG-11

FBD(5) sequence, as overhang;  
 Codon numbers (cf. Fig.6)  
 are indicated

sequence hybridizing with  
 SK gene; codon No.'s (Cf.  
 Fig.3) are indicated

	5                      7                      9
5'-TTC-ACC-GAT-GTT-CGT - ATT-GCT-GGA-CCT-GAG-TGG-CTG-CTA-GAC-3'	
255            257 259    1    3            5                      7                      9	

(SEQ ID NO:23)

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Paragraph beginning at page 49, line 29, has been amended as follows:

Upstream PCR-II primer, KRG-12

5'-TGG-TTT-TGA-TTT-TGG-ACT-TAA-GCC-TTG-3' (SEQ ID NO:24)

62            60    58            56            54

Note: sequence hybridizing with SK gene (codon No.'s are indicated; see Fig. 3)